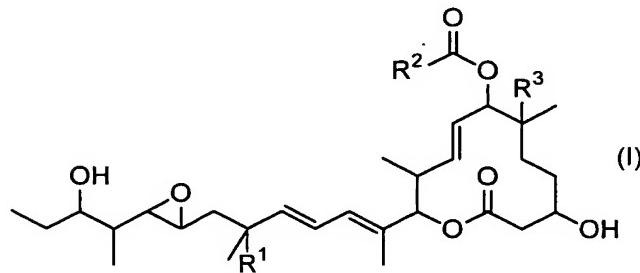


Claims

1. An assay method to predict sensitivity of a cancer cell to a compound represented by the following formula I, comprising using any one index of:

- 1) expression of pRB is reduced;
- 2) p16 is expressed;
- 3) expression of cyclin E is enhanced;
- 4) expression of pRB is reduced and expression of cyclin E is enhanced; or
- 5) p16 is expressed and expression of cyclin E is enhanced;

Formula (I)



(Wherein, R¹ represents

- (1) hydrogen atom or
- (2) a hydroxyl group;

R³ represents

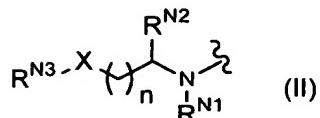
- (1) hydrogen atom,
- (2) a hydroxyl group or
- (3) a C₁₋₆ alkoxy group; and

R² represents

- (1) hydrogen atom,

(2) a C₁₋₆ alkyl group which may have a substituent,
(3) a C₇₋₁₀ aralkyl group which may have a substituent,
(4) a 5-membered to 14-membered heteroaralkyl group
which may have a substituent,

(5) the formula (II):



(wherein,

A)

n represent an integer of 0 to 4;

X represents

i) -CHR^{N4}-,

ii) -NR^{N5}- or

iii) -O-;

R^{N1} and R^{N2} are the same as or different from each other
and each represents

i) hydrogen atom or

ii) a C₁₋₆ alkyl group;

R^{N3} and R^{N4} are the same as or different from each other
and each represents

i) hydrogen atom,

ii) a C₁₋₆ alkyl group which may have a substituent,

iii) an unsaturated C₂₋₁₀ alkyl group which may have
a substituent,

iv) a C₁₋₆ alkoxy group which may have a substituent,

v) a C₆₋₁₄ aryl group which may have a substituent,

- vi) a 5-membered to 14-membered heteroaryl group which may have a substituent,
 - vii) a C₇₋₁₀ aralkyl group which may have a substituent,
 - viii) a C₃₋₈ cycloalkyl group which may have a substituent,
 - ix) a C₄₋₉ cycloalkylalkyl group which may have a substituent,
 - x) a 5-membered to 14-membered heteroaralkyl group which may have a substituent,
 - xi) a 5-membered to 14-membered non-aromatic heterocyclic group which may have a substituent,
 - xii) -NR^{N6}R^{N7} (wherein, R^{N6} and R^{N7} are the same as or different from each other and each represents hydrogen atom or a C₁₋₆ alkyl group) or
 - xiii) R^{N3} and R^{N4} are bound together with the carbon atom to which they are bound to form a 5-membered to 14-membered non-aromatic heterocyclic group which may have a substituent (the non-aromatic heterocyclic group may have a substituent);
- R^{N5} represents
- i) hydrogen atom,
 - ii) a C₁₋₆ alkyl group which may have a substituent,
 - iii) an unsaturated C₂₋₁₀ alkyl group which may have a substituent,
 - iv) a C₆₋₁₄ aryl group which may have a substituent,
 - v) a 5-membered to 14-membered heteroaryl group which

may have a substituent,

vi) a C₇₋₁₀ aralkyl group which may have a substituent,

vii) a C₃₋₈ cycloalkyl group which may have a substituent,

viii) a C₄₋₉ cycloalkylalkyl group which may have a substituent,

ix) a 5-membered to 14-membered heteroaralkyl group which may have a substituent,

x) a 5-membered to 14-membered non-aromatic heterocyclic group which may have a substituent, or

xi) R^{N3} and R^{N5} are bound together with the nitrogen atom to which they are bound to form a 5-membered to 14-membered non-aromatic heterocyclic group which may have a substituent (the non-aromatic heterocyclic group may have a substituent),

B)

X, n, R^{N3}, R^{N4} and R^{N5} represent the above defined groups; and R^{N1} and R^{N2} represent a 5-membered to 14-membered non-aromatic heterocyclic group which R^{N1} and R^{N2} are bound together to form and which may have a substituent,

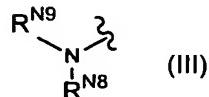
C)

X, n, R^{N2}, R^{N4} and R^{N5} represent the above defined groups, and R^{N1} and R^{N3} represent a 5-membered to 14-membered non-aromatic heterocyclic group which R^{N1} and R^{N3} are bound together to form and which may have a substituent, or

D)

X, n, R^{N1}, R^{N4} and R^{N5} represent the above defined groups; and R^{N2} and R^{N3} represent 5-membered to 14-membered non-aromatic heterocyclic group which R^{N2} and R^{N3} are bound together to form and which may have a substituent), or

(6) the formula (III):



(wherein, R^{N8} and R^{N9} are the same as or different from each other and each represents

- i) hydrogen atom,
- ii) a C₁₋₆ alkyl group which may have a substituent,
- iii) a C₆₋₁₄ aryl group which may have a substituent,
- iv) a 5-membered to 14-membered heteroaryl group which may have a substituent

v) a C₇₋₁₀ aralkyl group which may have a substituent,
or

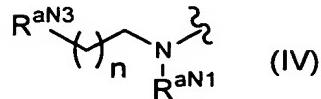
vi) a 5-membered to 14-membered heteroaralkyl group
which may have a substituent)).

2. The assay method according to claim 1, wherein R² is

- 1) hydrogen atom;
- 2) a C₁₋₆ alkyl group which may have a substituent,
- 3) a C₇₋₁₀ aralkyl group which may have a substituent
or
- 4) a 5-membered to 14-membered heteroaralkyl group
which may have a substituent.

3. The assay method according to claim 1, wherein R² is represented by the following formula (IV):

Formula (IV)



(wherein n represents an integer of 0 to 4;
R^{aN1} represents

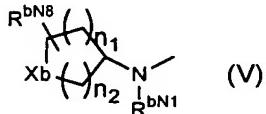
- (1) hydrogen atom or
- (2) a C₁₋₆ alkyl group;

R^{aN2} represents

- (1) hydrogen atom
- (2) a N-C₁₋₆ alkylamino group,
- (3) a N,N-di-C₁₋₆ alkylamino group,
- (4) ethylmethylamino group,
- (5) pyridyl group,
- (6) pyrrolidin-1-yl group,
- (7) piperidin-1-yl group,
- (8) morpholin-4-yl group or
- (9) 4-methylpiperazin-1-yl group).

4. The assay method according to claim 1, wherein R² is represented by the following formula (V):

Formula (V)



(wherein n₁ and n₂ are the same as or different from each other and each represents an integer of 0 to 4;

X_b represents

- 1) $-\text{CHR}^{bN4}-$,
- 2) $-\text{NR}^{bN5}-$ or
- 3) $-\text{O}-$;

R^{bN1} represents

- 1) hydrogen atom or
- 2) a C_{1-6} alkyl group;

R^{bN8} represents

- 1) hydrogen atom,
- 2) a C_{1-6} alkyl group,
- 3) a C_{6-14} aryl group or
- 4) a C_{7-10} aralkyl group;

R^{bN4} represents

- 1) hydrogen atom,
- 2) a C_{1-6} alkyl group which may have a substituent,
- 3) an unsaturated C_{2-10} alkyl group which may have a substituent,
- 4) a C_{1-6} alkoxy group which may have a substituent,
- 5) a C_{6-14} aryl group which may have a substituent,
- 6) a 5-membered to 14-membered heteroaryl group which may have a substituent,
- 7) a C_{7-10} aralkyl group which may have a substituent,
- 8) a C_{3-8} cycloalkyl group which may have a substituent,
- 9) a C_{4-9} cycloalkylalkyl group which may have a substituent,
- 10) a 5-membered to 14-membered heteroaralkyl group

which may have a substituent,

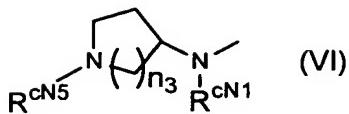
11) $-NR^{bN6}R^{bN7}$ (wherein R^{bN6} and R^{bN7} are the same as or different from each other and each represents hydrogen atom or a C₁₋₆ alkyl group) or

12) a 5-membered to 14-membered non-aromatic heterocyclic group which may have a substituent; and R^{bN5} is

- 1) hydrogen atom,
- 2) a C₁₋₆ alkyl group which may have a substituent,
- 3) an unsaturated C₂₋₁₀ alkyl group which may have a substituent,
- 4) a C₆₋₁₄ aryl group which may have a substituent,
- 5) a 5-membered to 14-membered heteroaryl group which may have a substituent
- 6) a C₇₋₁₀ aralkyl group which may have a substituent,
- 7) a C₃₋₈ cycloalkyl group which may have a substituent,
- 8) a C₄₋₉ cycloalkylalkyl group which may have a substituent,
- 9) a 5-membered to 14-membered heteroaralkyl group which may have a substituent or
- 10) a 5-membered to 14-membered non-aromatic heterocyclic group which may have a substituent).

5. The assay method according to claim 1, wherein R² is represented by the following formula (VI):

Formula (VI)



(wherein n_3 represents an integer of 1 or 2;

R^{cN1} represents

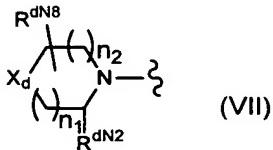
- (1) hydrogen atom or
(2) a C₁₋₆ alkyl group;

R^{cN5} represents

- (1) hydrogen atom or
(2) a C₁₋₆ alkyl group).

6. The assay method according to claim 1, wherein R² is represented by the following formula (VII):

Formula (VII)



(wherein n_1 and n_2 are the same as or different from each other
and each represents an integer of 0 to 4;

X_d represents

- 1) $-\text{CHR}^{\text{dN4}}-$,
 - 2) $-\text{NR}^{\text{dN5}}-$ or
 - 3) $-\text{O}-$; and

R^{dN2} represents

- 1) hydrogen atom or
 - 2) a C₁₋₆ alkyl group;

R^{dN8} represents

- 1) hydrogen atom,

- 2) a C₁₋₆ alkyl group,
- 3) a C₆₋₁₄ aryl group or
- 4) a C₇₋₁₀ aralkyl group;

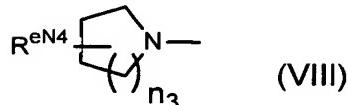
R^{dN4} represents

- 1) hydrogen atom,
 - 2) a C₁₋₆ alkyl group which may have a substituent,
 - 3) an unsaturated C₂₋₁₀ alkyl group which may have a substituent,
 - 4) a C₁₋₆ alkoxy group which may have a substituent,
 - 5) a C₆₋₁₄ aryl group which may have a substituent,
 - 6) a 5-membered to 14-membered heteroaryl group which may have a substituent,
 - 7) a C₇₋₁₀ aralkyl group which may have a substituent,
 - 8) a C₃₋₈ cycloalkyl group which may have a substituent,
 - 9) a C₄₋₉ cycloalkylalkyl group which may have a substituent,
 - 10) a 5-membered to 14-membered heteroaralkyl group which may have a substituent,
 - 11) -NR^{dN6}R^{dN7} (wherein R^{dN6} and R^{dN7} are the same as or different from each other and each represents hydrogen atom or a C₁₋₆ alkyl group) or
 - 12) a 5-membered to 14-membered non-aromatic heterocyclic group which may have a substituent; and
- R^{dN5} represents
- 1) hydrogen atom,
 - 2) a C₁₋₆ alkyl group which may have a substituent,

- 3) an unsaturated C₂₋₁₀ alkyl group which may have a substituent,
- 4) a C₆₋₁₄ aryl group which may have a substituent,
- 5) a 5 to 14-membered ring heteroaryl group which may have a substituent,
- 6) a C₇₋₁₀ aralkyl group which may have a substituent,
- 7) a C₃₋₈ cycloalkyl group which may have a substituent,
- 8) a C₄₋₉ cycloalkylalkyl group which may have a substituent,
- 9) a 5-membered to 14-membered heteroaralkyl group which may have a substituent or
- 10) a 5-membered to 14-membered non-aromatic heterocyclic group which may have a substituent).

7. The assay method according to claim 1, wherein R² is represented by the following formula (VIII):

Formula (VIII)



(wherein n₃ represents an integer of 1 to 3; and

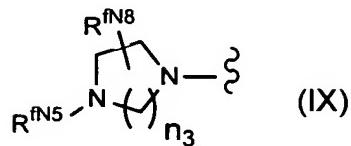
R^{eN4} represents

- (1) amino group,
- (2) a N-C₁₋₆ alkylamino group,
- (3) pyrrolidin-1-yl group,
- (4) piperidin-1-yl group or
- (5) morpholin-4-yl group).

8. The assay method according to claim 1, wherein R²

is represented by the following formula (IX) :

Formula (IX)



(wherein n₃ represents an integer of 1 to 3;

R^{fN8} represents

- 1) hydrogen atom,
- 2) a C₁₋₆ alkyl group,
- 3) a C₆₋₁₄ aryl group or
- 4) a C₇₋₁₀ aralkyl group; and

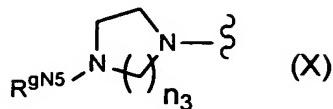
R^{fN5} represents

- 1) hydrogen atom,
- 2) a C₁₋₆ alkyl group which may have a substituent,
- 3) a C₃₋₈ cycloalkyl group which may have a substituent,
- 4) a 3-membered to 8-membered ring nonaromatic heterocyclic group which may have a substituent,
- 5) a C₆₋₁₄ aryl group which may have a substituent,
- 6) a 5-membered to 14-membered heteroaryl group which may have a substituent,
- 7) a C₇₋₁₀ aralkyl group which may have a substituent,
- 8) a 5-membered to 14-membered heteroaralkyl group which may have a substituent or
- 9) a C₄₋₉ cycloalkylalkyl group which may have a substituent).

9. The assay method according to claim 1, wherein R²

is represented by the following formula (X):

Formula (X)



(wherein n₃ represents an integer of 1 to 3; and

R^{gN5} represents

- 1) hydrogen atom
- 2) a C₁₋₆ alkyl group which may be substituted,
- 3) a C₃₋₈ cycloalkyl group which may be substituted,
- 4) a C₄₋₉ cycloalkylalkyl group which may be substituted,
- 5) a C₇₋₁₀ aralkyl group which may be substituted,
- 6) a pyridyl group which may be substituted or
- 7) a tetrahydropyranyl group which may be substituted).

10. The assay method according to claim 1, wherein the compound represented by the formula (I) is any one compound selected from the group consisting of the following compounds:

- 1) (8E,12E,14E)-7-acetoxy-3,6,21-trihydroxy-6,10,12,16,20-pentamethyl-18,19-epoxytricosane-8,12,14-trien-11-olide,
- 2) (8E,12E,14E)-7-((4-cycloheptylpiperazin-1-yl)-carbonyl)oxy-3,6,16,21-tetrahydroxy-6,10,12,16,20-pentamethyl-18,19-epoxytricosane-8,12,14-trien-11-olide,
- 3) (8E,12E,14E)-3,6,16,21-tetrahydroxy-7-((4-isopropylpiperazin-1-yl)carbonyl)oxy-6,10,12,16,20-pentamethyl-18,19-epoxytricosane-8,12,14-trien-11-olide;

and

4) (8E,12E,14E)-3,6,16,21-tetrahydroxy-6,10,12,16,20-pentamethyl-7-((4-methylpiperazin-1-yl)carbonyl)oxygen-18,19-epoxytricosane-8,12,14-trien-11-oxide.

11. The assay method according to claim 1, comprising assaying a reduced expression of pRB, an expression of p16 or an enhanced expression of cyclin E by measuring the levels of their respective encoding mRNAs.

12. The assay method according to claim 11, wherein the method for measuring the level of the mRNAs is a quantitative RT-PCR method.

13. The assay method according to claim 11, wherein the method for measuring the level of the mRNAs is a DNA tip method.

14. The assay method according to claim 1, comprising assaying a reduced expression of pRB, an expression of p16 or an enhanced expression of cyclin E by measuring the levels of their respective proteins.

15. The assay method according to claim 14, wherein the method for measuring the levels of their respective proteins is a western blot method.

16. The assay method according to claim 14, wherein the method for measuring the levels of their respective proteins is an immunohistostaining method.

17. The assay method according to claim 14, wherein the method for measuring the levels of their respective proteins

is an ELISA method.

18. A kit for use in the assay method according to 12, comprising a primer that contains at least 15 consecutive base sequences of the pBR, p16 or cyclin E genes.

19. A kit for use in the assay method according to 15, 16 or 17, comprising an antibody to the pRB, p16 or cyclin E.